REMARKS

Applicants appreciatively acknowledge that the Examiner has withdrawn a number of prior rejections. The Examiner maintains some objections and introduces some new rejections which are listed here in the order in which they are addressed:

1. The Examiner objects to the specification as allegedly "incorporating by reference" through web links.

2. Claims 1-8, 10-12, 14-22, 24-26, 28, 35-37, 39-46 and 48-50 are rejected under 35 U.S.C. § 112 ¶ 2, as being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention.

3. The Claims are rejected under 35 U.S.C. § 103(a) as being obvious.

1. There Is No Incorporation By Reference

The Examiner objects to the specification as allegedly "incorporating by reference" through web links. The Examiner points to page 22 (line 18) of the specification, by way of an example. However, it appears that the Examiner has read more into the text than is there. The actual text reads:

> "Exemplary chemicals are described at http://dir.niehs.nih.gov/dirtb/dirrtg/chemicalsstudiedindex2.htm including, but not limited to, *N*-ethyl-*N*-nitrosurea (ENU), methylnitrosourea (MNU), procarbazine hydrochloride (PRC), triethylene melamine (TEM), acrylamide monomer (AA), chlorambucil (CHL), melphalan (MLP), cyclophosphamide (CPP), diethyl sulfate (DES), ethyl methane sulfonate (EMS), methyl methanes ulfonate (MMS), 6-mercaptopurine (6MP), mitomycin-C (MMC), procarbazine (PRC), *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), ^3H_2O, and urethane (UR) [see, *e.g.*, Russell *et al.*, Factors affecting the nature of induced mutations, In "Biology of Mammalian Germ Cell Mutagenesis," Banbury Report 34, Cold Spring Harbor Laboratory Press (1990), pp. 271-289; Rinchik (1991) Trends in Genetics 7(1); Marker *et al.* (1997) Genetics 145:435-443]."

The Examiner is requested to take note that the phrase "incorporated by reference" does not appear in the above-quoted text. Moreover, rather than being an incorporation by reference, the text lists the names and/or formulas for the various chemicals. Finally, a number of publications are cited in the same context. Thus, the Examiner proceeds from an incorrect assumption, namely that any "incorporating by reference" is intended. The specification merely takes note of a helpful website. Applicants therefore request that the objection be withdrawn.

2. The Claims Are Not Indefinite According To 35 U.S.C. § 112 ¶ 2

Claims 1-8, 10-12, 14-22, 24-26, 28, 35-37, 39-46 and 48-50 are rejected under 35 U.S.C. § 112 ¶ 2, as being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. Applicants cannot agree.

A. Claims 1-36

The Examiner argues that Claims 1, 15, 35 and 36 are vague because "there is no specific . . . method of isolation recited." Applicants respectfully submit that this is not a proper 112 rejection.

The Examiner is reminded that the claims are not to be read out of context. Rather, they are to be read in the context of the specification. There a number of procedures described in the specification which explain how cells can be isolated (e.g. subculturing, drug selection, etc.). Since the specification makes clear what methods can be used for isolating, such methods need not be included in the claims. The claims do not need to elaborate on specific methods or protocols.

Nonetheless, Applicants elect at this time to cancel Claims 1-8, 10-12, 14-22, 24-26, 28, and 35-36. The cancellation of these claims is done without acquiescing to the Examiner's rejection, but rather to further the prosecution. Applicants hereby expressly reserve the right to prosecute the cancelled claims (or similar claims) in the future.

B. Claims 37-50

The Examiner argues with respect to Claim 37 that "it is unclear if 70% of the time the gene of interest has an alteration or if 70% of the genes in the genome are altered." In fact, the specification makes clear that a "statistical probability" is intended in the context of a modification of all of the genes in the genome:

> ". . . the statistical probability, preferably at least about 70% probability, more preferably at least about 85% probability, and most preferably at least about 95% probability, as determined by a standard Poisson distribution, that each gene in the genome contains at least one modification."

(*see* specification, page 14). Without acquiescing to the Examiner's rejection, but to further the prosecution by clarifying one embodiment of the present invention, Applicants have amended Claims 37, 39 and 40. It is submitted that the amendments moot the rejection.

With respect to Claim 41, the Examiner's assumption as to the number of cells required (see page 6 of the Office Action) is not correct. Using fewer cells simply means

conditions must be used wherein a greater number of modifications per cell are achieved. The Examiner is requested to note that nothing in Claim 37 indicates that only one modification of one gene is achieved per one cell.

With respect to the "gene of interest," it is clear (from the claims themselves and the specification) that "every gene" is a term which includes the "gene of interest." In Claim 37, while many genes are modified, the selection step is based on changes to the "gene of interest." Therefore, the rejection is unfounded.

3. The Claims are not Obvious

The Claims are rejected under 35 U.S.C. § 103(a) as being obvious. The Examiner has made an obviousness rejection based on the single reference of Schafer et al. The Examiner has also made an obviousness rejection based on the single reference of Goodfellow et al. Finally, the Examiner maintains an obviousness rejection based on a combination of references: Shafer, Goodfellow, in view of Kohler or Guay-Woodford.

A. Schafer Teaches Away

As noted above, certain claims have been cancelled for other reasons (at this time) rendering the obviousness rejection as to these claims moot. Claim 37 requires:

> "treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) it is at least 70% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest . . ."

At no point does the Examiner even address this limitation. The Examiner is reminded that an obviousness rejection must show how <u>all</u> of the elements are taught to be used in the method claimed. This has not been done.

While it is not Applicants' burden to go through the evidence (particularly when the Examiner has not addressed all of the claim limitations), to further the prosecution the Applicants respectfully note that the Schafer patent refers to "one mutation in every 1,000 genes." (col. 2, line 10). Indeed, Schafer teaches that "a large number of mutations per individual is undesirable . . ." (col. 4, line 55). Instead of working with conditions which generate a high number of modifications, Schafer teaches the use of a larger population ("a

larger population . . . is mutated and screened." (col 4., lines 66-67). Thus, Schafer can be said to teach away from conditions where "at least one modification in substantially every gene . . . is produced" in a relatively small number of embryonic cells.[1]

B. Goodfellow Teaches Away

The Examiner, in making the obviousness rejection based on the Goodfellow reference, makes the following argument about the 70% element in Claim 37: "a fair interpretation is that 70% of the cells in the culture have a random genetic alteration." Whether or not that was a "fair interpretation" for Claim 37 before this response, Claim 37 has been amended (in accordance with the language of the specification) such that it is not a "fair interpretation" at this point. There is no indication in Claim 37 that a "per cell" limitation is intended.

When Goodfellow is re-examined, it is clear that the "1 mutation occurs in every 10,000-1,000 genes" cited by the Examiner (see Office Action, p. 10) puts Goodfellow in the same position as Schafer, i.e. it teaches away. Goodfellow certainly does not teach the use of conditions where "it is at least 70% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced" in a relatively small collection of ES cells.

C. The Examiner Must Provide Evidence

The Examiner admits that Goodfellow "does not specifically recite the use of a fluorescent chemical cleavage method . . . " (Office Action, p. 14). The Examiner nonetheless argues that "it [is] generally taught that any method convention in the art can be used." The Examiner has also cited case law in response to applicants argument that there is no basis for combining the prior art references. Specifically, the Examiner cites *In re McLaughlin* for the proposition that one can consider the references collectively to determine what the combination "taken as a whole" would have suggested to one skilled in the art. The

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[1] "A reference may be said to teach away when a person of ordinary skill, upon [examining] (sic) the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *Para-Ordnance Manufacturing v. SGS Importers International*, 37 USPQ2d 1237,1241 (Fed. Cir. 1995) (quoting *In re Gurley*, 31 USPQ2d 1130, 1131 (Fed. Cir. 1994)).

Examiner cites *In re Nilssen* for the proposition that the Examiner need not point to something within the references themselves to support the combination. It is respectfully submitted that the Examiner's cases i) do not stand for the propositions asserted by the Examiner, and/or ii) do not reflect the current state of the law.

i. The Examiner Misunderstands the *McLaughlin* case

Applicants have argued that the Examiner is improperly considering the references collectively **before** establishing the threshold requirement that a person skilled in the art would be motivated to combine these references in the first place. The Examiner justifies skipping this threshold requirement by citing *In re McLaughlin* - a case over thirty years old that predates the Federal Circuit. The Examiner is requested to take note that the *McLaughlin* case involved a situation where the references combined with the primary reference were found to suggest the use of side panels with the loads of the primary reference. No such "suggestion" in Kohler or Guay-Woodford is pointed to by the Examiner.

Moreover, modern case law from the Federal Circuit makes it clear that the references cannot be considered **collectively** until the Examiner points to some motivation to combine these references in the first place. The purpose of this threshold requirement is to prevent the Examiner from using the invention itself and hindsight reconstruction to defeat the patentability of the invention. The Federal Circuit, in a recent decision, articulates this position, stating:

> To prevent the use of hindsight based on the invention to defeat patentability of the invention, this court requires the examiner to show a motivation to combine the references that create the case of obviousness. In other words, the examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed. *In re Rouffet et al.,* 149 F.3d 1350, 1357 (Fed. Cir., 1998).

The Examiner does not 'show reasons' why one skilled in the art would be motivated to combine these reference, but instead merely points to the desirability of the claimed combination. As such, he has failed to satisfy this threshold requirement. In light of this discussion, the Examiner's citation to *In re McLaughlin* is premature and not applicable to this case where no motivation to combine has been established.

ii. The Examiners Reliance on *Nilssen* is Misplaced

The Examiner has cited the *In re Nilssen* case to support an argument that the Examiner need not point to something in the references themselves to support the combination. The Examiner's reliance on *Nilssen* is misplaced.

The requirement that the Examiner make a showing of a suggestion, teaching or motivation to combine the prior art references is "an essential evidentiary component of an obviousness holding." *C.R. Bard, Inc. v. M3 Sys. Inc.*, 157 F.3d 1340, 1352 (Fed. Cir. 1998). There are three sources for this evidentiary component: the prior art references themselves, the knowledge of one of ordinary skill in the art, or, in some cases, from the nature of the problem to be solved. *Pro-Mold & Tool Co. v. Great Lakes Plastics, Inc.*, 75 F.3d 1568, 1573 (Fed. Cir. 1996). The suggestion most often comes from the teachings of the pertinent references. *In re Rouffet*, 149 F.3d 1350, 1359 (Fed. Cir. 1998). Nonetheless, regardless of the source of the requisite evidence, the Examiner's showing "must be clear and particular, and broad conclusory statements about the teaching of multiple references, standing alone, are not 'evidence'." *In re Dembiczak*, 175 F.3d 994, 1000 (Fed. Cir. 1999).

The *In re Nilssen* case merely underscores the point that the evidence need not come expressly from the references in all cases.[2] The case does NOT change the fact that it is the Examiner's burden to present "evidence" for the combination and that this showing must be "clear and particular." Importantly, since an Examiner is NOT one skilled in the art (under the law), the Examiner's opinion on what one skilled in the art might believe does not count. *In re Rijckaert*, 9 F.3d 1531, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993) ("[T]he examiner's assumptions do not constitute the disclosure of the prior art.").

Of course, if the Examiner has knowledge of relevant facts which are used to make the rejection, the Examiner is free to use those facts - but only if submitted in the form of an affidavit. *See* 37 CFR 1.107(b). In the present case, the Examiner has submitted no such affidavit.

Indeed, the Examiner has provided only the Examiner's opinion and conclusory statements - this is not the requisite "evidence" needed to support the combination. The Examiner simply asserts - without a basis - that "it would be obvious" to use any technique

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[2] For example, in litigation evidence can be submitted through experts on this question.

(such as fluorescent cleavage mismatch) and any disease related gene (such as p53 and PKD3). The above-cited case law shows that this is not adequate and the Examiner has not satisfied the requirements for combining the art.

Finally, even if the art is (improperly) combined, the Examiner must find the limitation regarding conditions of high modification (discussed above) which is now clearly part of Claim 37. None the references provide this element. Therefore, the rejection must be withdrawn.

CONCLUSION

Applicants believe that the arguments set forth above traverse the Examiner's rejections and therefore request that these grounds for rejection be withdrawn for the reasons set forth above. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned collect at (617)-252-3353.

Dated: April 17, 2003

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APPENDIX I
MARKED-UP VERSION OF REWRITTEN CLAIMS
PURSUANT TO 37 CFR § 1.121 (c)(1)(ii)

37. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) <u>it is at least 70% probable that</u> at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest[, wherein said treating is under conditions such that at least one modification in at least 70% of the genes in said mouse embryonic stem cells is produced]; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

39. (Amended) The method of Claim 37, wherein said treating is under conditions such that <u>it is</u> at least [one modification in at least] 85% [of the genes] <u>probable that at least one modification in substantially every gene</u> in said mouse embryonic stem cells is produced.

40. (Amended) The method of Claim 37, wherein said treating is under conditions such that it is at least [one modification in at least] 95% [of the genes] probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

46. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) *N*-ethyl-*N*-nitrosourea;

b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embyronic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9000;

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells; and

d) detecting at least one of said first and second modifications in said gene of interest using fluorescent chemical cleavage of mismatch.

APPENDIX II
CLEAN VERSION OF THE ENTIRE SET OF PENDING CLAIMS
PURSUANT TO 37 CFR § 1.121 (c)(3)

37. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) it is at least 70% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

39. (Amended) The method of Claim 37, wherein said treating is under conditions such that it is at least 85% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

40. (Amended) The method of Claim 37, wherein said treating is under conditions such that it is at least 95% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

41. The method of Claim 37, wherein the number of said isolated mouse embryonic stem cells in said in vitro culture consists of from 200 to 600 embryonic stem cells, and said chemical agent is N-ethyl-N-nitrosourea.

42. The method of Claim 37, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

43. The method of Claim 37, wherein said gene of interest is associated with a disease.

44. The method of Claim 43, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

45. The method of Claim 37, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

46. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) *N*-ethyl-*N*-nitrosourea;

b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embyronic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells cells having a first modification in said gene of interest, and embryonic stem cells having a second

modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9000;

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells; and

d) detecting at least one of said first and second modifications in said gene of interest using fluorescent chemical cleavage of mismatch.

48. The method of Claim 46, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

49. The method of Claim 46, wherein said gene of interest is associated with a disease.

50. The method of Claim 49, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

51. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising between 200 and 600 isolated mouse embryonic stem cells, each of said cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) it is at least 70% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

52. (New) The method of Claim 51, wherein said treating is under conditions such that it is at least 85% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

53. (New) The method of Claim 51, wherein said treating is under conditions such that it is at least 95% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

54. (New) The method of Claim 51, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

55. (New) The method of Claim 51, wherein said gene of interest is associated with a disease.

56. (New) The method of Claim 55, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

57. (New) The method of Claim 51, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.